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Dinotefuran and Piperonyl Butoxide Mixture for The Extermination and Prevention of Ctenocephalides Canis and Ctenocephalides Felis Felis In Dogs And Cats

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Abstract--- *Ctenocephalides canis* and *Ctenocephalides felis felis* are insects which are among the most common ectoparasites of common household dogs and cats. Flea killers have been developed for decades to counter this worldwide pest. It is a never-ending battle because of the continuous genetic resistance presented by the pest. In the present study, we applied a mixture of the pesticide Dinotefuran (26% w/v) and the synergist Piperonyl butoxide (6% w/v) to dogs (Solpreme Dog™ and cats (Solpreme Cat™) infested with the fleas via "Spot on" method. The treatment exterminated all of the fleas within 3 days (100%, p< 0.001). Re-infestation after 10 days caused an increase in the number of fleas, yet most of the effect lasted for at least 30 days. Further tests showed that the treatment is safe, water resistant, and has a long shelf life. The development of this novel mixture of substances provides an effective, safe means in the struggle with the continuous development of resistance to pesticides among major skin parasites.

***Index Terms*---cats, Ctenocephalides canis and felis, Dinotefuran, Dogs, Piperonyl butoxide.**

I. INTRODUCTION

Ctenocephalides canis and *Ctenocephalides felis felis* are insects which are among the most common ectoparasites of dogs and cats [1]. Their distribution is global and is magnified by the fact that their hosts regularly live in close proximity with human. Fleas present serious medical issues to also human due to their ability to carry and transmit diseases through blood by biting their hosts. In addition to the direct action they possess as ectoparasites, they exert an indirect action as well by transferring numerous endosymbionts or pathogens including bacteria, protozoa and helminths that they carry, either directly or as intermediate hosts [2]. Another significant economic factor is that Ctenocephalides have an ability to develop resistance to pesticides, necessitating a constant effort to develop new products. The present study was designed to test the efficacy of a mixture containing dinotefuran and piperonyl butoxide, in concentrations developed at the laboratories of Solano (Solpreme Dog™ and Solpreme Cat™), for the extermination of these skin parasites in dogs and cats.

Dinotefuran is a neonicotinoid belonging to the newer generations of pesticides found effective to control a wide range of pests in agriculture [3]. Dinotefuran binds to a specific site on the insect cholinergic receptor, different than the site where the neonicotinoids normally bind. The binding is very long lasting causing unceasing nerve stimulation making it a fast and effective insecticide [4].

Piperonyl butoxide (PBO) is an insecticide synergist, a chemical that is used to enhance the potency of insecticides [5]. PBO acts as a synergist by inhibiting the P450 enzymes which break down the insecticides, prolonging their activity [6]. Today, dinotefuran and piperonyl butoxoide are being used on pets but have not been used together in the same product until now. We have hypothesized that because of their efficacy and their known safety of margin, they could act synergistically in the mixture developed in Solano laboratories to become effective and safe spot-on drops for the treatment against Ctenocephalides in dogs and cats.

There is a constant need to develop new generations of pesticides because of the increase of both toxicity to the parasite and safety for the host, human, other organisms, and the environment, and also to overcome resistance if developed. The success of an adulticide is by nature time limited because mutations for resistance to the substance quickly proliferate in the pest population. This major issue [1] was brought to light as early as the forties in relation to the diminishing effectiveness of DDT in the extermination of Pulex irritans [7]. Since then, resistance of *Ctenocephalides felis* to various levels organophosphates, pyrethrins, pyrethroids, carbamates, and fipronil has been reported [8-10]. Similarily, the use of Sevin, with the major active ingredient carbaryl is limited today because of its diminishing efficacy. Thus, the issue of countering developing genetic resistance to pesticides is central to pesticide control [11], and to date the most effective mean to cope with this challenge is the continuous development of new products. In this light, it is significant that dinotefuran maintains its efficacy, and notably, no resistance to date has developed [12].

Consequently, in the present study dogs and cats were infested with adult *Ctenocephalides canis* and *felis* fleas and were then treated with Dinotefuran (26% w/v) and Piperonyl butoxide (6% w/v) mixture (Solpreme Dog™ and Solpreme Cat™) by "Spot on" application. The efficacy of the treatment was assessed by parasite counting. The practicality of the substance in daily situations was confirmed by studying its efficacy in dogs that were wetted and by confirming its shelf life over a reasonable period.

II. METHODS

Dog study

Animals and maintenance: The experiment used 47 dogs 8 weeks to 12 years old, weighing from about 1.5 kg to 50 kg. Animals were housed in groups of 4 in 20m^2 pens. They were fed with standard commercial dry dogs' diet; water was provided ad lib. The dogs were acclimated for 10 days prior to the initiation of the study. The candidate animals were

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examined by a veterinarian and their suitability was ascertained prior to inclusion in the study. Animals were free of infectious diseases, which could complicate the interpretation of the study results. Only animals in good health conditions were enrolled for the study. The study lasted for 30 days. **Flea infestation:** A mix of adult *Ctenocephalides canis* and *felis* fleas originating from dogs were collected and kept at 5-8 °C. Fifty fleas were placed in the groin, back and behind the ears of the dogs. After the body temperature of the fleas rose to a normal level they began to actively bite their hosts. *Monitoring infestation and clinical signs:* The level of infestation was monitored during the entire period of the experiment. All dogs were observed daily for the physical condition of their hair and for tangles between their shoulders. *Clinical observations of the dogs after infestation:* I. Veterinary observation: Changes in skin and fur, eyes and mucous membranes, respiratory system, circulatory system, autonomic and central nervous system, somatomotor activity, as well as behavior pattern were monitored. Particular attention was directed to observations of central nervous system signs (seizures, tremors and salivation), vomiting and diarrhea. II. Changes in body weight: Dogs were weighed at the beginning and end of the study. III. Blood tests: Blood samples for clinical tests were collected from the v. cephalica antebrachii before the first treatment (Test 1) and on day 30 (last day) of the study (Test 2). The parameters for the tests were urea, creatinine, bilirubin, *alkaline phosphatase (*ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST).

This study was based on EMEA/CVMP/EWP/005 "Guideline for the Testing and Evaluation of the Efficacy of Antiparasitic Substances for the Treatment and Prevention of Tick and Flea Infestation in Dogs and Cats", OPPTS 870.7200 “Companion Animal Safety”; and was performed with the approach to the OECD Principles of Good Laboratory Practice Directive 2004/10/EC and US FDA Good Laboratory Practice Regulations, 21 CFR Part 58. Ethical permit: License for Pharmaceutical Activities for Animal Studies No. 39-09-3-023, 08/06/2009

III. RESULTS

Global statistical analysis (ANOVA [13]) suggests that there are no statistical differences in dogs and cats regarding the level of infestation and the efficacy of eradication of the fleas across size, age and gender groups. Similarly, there were no statistical differences in the efficacy period between the different dog groups (no dog groups X efficacy interaction). Consequently, the results of the different sizes, ages and gender of the dogs and cats were pooled, and are presented and statistically analyzed accordingly.

Treatment of the flea infestation: The dogs in all study groups were treated after infestation with Dinotefuran (26% w/v) and Piperonyl butoxide (6% w/v) mixture (Solpreme Dog™). Drops were applied on the back of the neck (“Spot on” application), in doses based on preliminary studies according to dog’s weight, and were as follows – 4.0 ml for 25 – 50 kg body weight, 2.5 ml for 10 – 25 kg, 1.0 ml for 4 – 10 kg, and 0.4 ml for dogs and puppies weighing 1.5 – 4 kg.

Re-infestations with fleas: On the tenth day (D10) of the study re-infestations were performed to assess the duration of efficacy of the treatment.

Additional studies were conducted to establish certain properties of the anti-fleas mixture drops.

The shelf life of the drops: The efficacy of the drops was tested on three year old batches.

$$\text{Efficacy} = \frac{MFC_B - MFC_A}{MFC_B} \times 100\%$$

$_B$ = before treatment, $_A$ After treatment
MFC = Mean Fleas Count

Safety tests for the anti-fleas treatment mixture: a) A safety test of the anti-fleas mixture drops was conducted by treating a group of dogs and pups with 5 times higher and lower than the recommended dose. b) A safety test of the non-active ingredients was conducted with 5 times the recommended dose of control vehicle treatment that contains non-active, inert ingredients.

Testing the water resistant properties of the substance: The efficacy of the drops was tested in treated dogs which were washed once daily throughout the study

Evaluating the efficacy of the anti-fleas Solpreme treatment: Ectoparasite presence was monitored in animals in the efficacy test groups. Efficacy was monitored periodically throughout the entire test period. Efficacy was calculated as follows:

Cat study

The procedure was similar to the dogs’ as described above. Changes specific to the cat study will be detailed.

Animals and maintenance: The experiment used 47 cats and kittens 8 weeks to 12 years old, weighing from about 0.6 to 8 kg. Eight weeks is the first age where the kittens are no longer sensitive to the toxic effect of the substance. Animals were housed in groups of 4 in 20m^2 pens. They were fed with standard commercial dry cats’ diet; water was provided ad lib.

Doses: Adult cats weighing more than 4 kg were treated with 0.8 ml, kittens and small cats between 0.6 – 4 kg with 0.4 ml.

All the rest of the procedures and tests were similar to those of the dogs’ (above), except that **testing the water resistant properties of the substance and its shelf-life** were not conducted.

Ethical Standards

As is shown in **Table 1**, no more than 24 hours are required for the anti-fleas treatment mixture drops to have a significant effect, eradicating 94% of the original flea population in dogs ($P<0.001$ for the difference between the first day of infestation where the treatment was applied and the second day). In the cat, the respective difference was 99% ($P<0.001$). After 3 and 10 days the eradication in both species was complete (100%) and there was a partial recovery of the flea populations on days 20 and 30. Still, the number of the fleas was significantly lower ($P<0.001$) than that of the original population.

Table 1: I. The efficacy of Solpreme anti-flea mixture on eradicating the fleas in dogs and cats after infestation: percent eradication on different days after infestation (formula in Materials and methods), II. Comparison between dogs and cats infested with fleas (infested) with their respective infested groups treated with Solpreme (Infested-Treated). Numbers are means±SEM of the percent eradication a day after infestation and in various intervals during the next 30 days.

Day	Infested (4)	Infested-Treated (20)
	DOGS	
1	62±15	94±0.2 ***
3	77±14	100±0 ***
10[a]	46±16	100±0 *** ††
20	31±13	99±1 *** ††
30	0±0	87±4 *** †††
	CATS	
1	10±6	99±0.6 *** †††
3	23±9	100±0 *** †††
10[a]	34±12	100±0 *** †††
20	35±10	97±1 *** †††
30	8±8	94±2 *** †††

[a]The animals were re-infested after counting on day 10.
$P<0.001$ for the global treatment effect.
*** $P<0.001$ for the statistical significance (multiple level ANOVA, match pairs) of the difference from day 0 (the day of infestation).
†† $P<0.01$, ††† $P<0.001$, for the statistical significance of the difference between the Infested and the respected Infested-Treated scores

As is shown in **Table 2.** Long term storage for three years (shelf life) did not at all diminish the efficacy of Solpreme. Table 2. Additionally demonstrates the washing resistance of the of Solpreme anti-flea mixture. The dogs that were infested and treated with old Solpreme batch (Infested-Treated Old of Table 2) were also washed daily as described in Material and Methods. Washing the dogs (not done on the cats) had no effect on the efficacy of the anti-fleas mixture as their scores did not differ from those of the respective unwashed dogs.

Table 2: The effect of shelf life on the efficacy of Solpreme: Comparison between animal infested with fleas and treated with the new Solpreme mixture (Infested-Treated New) with their respective animals infested and treated Solpreme produced three years before (Infested-Treated Old).

Day	Infested-Treated New[b] (20)	Infested-Treated Old (4)
	DOGS	
1	62±15	90±5
3	77±14	100±0
10[a]	46±16	100±0
20	31±13	94±4
30	0±0	68±9
	CATS	
1	10±6	99±1
3	23±9	100±0
10[a]	34±12	100±0
20	35±10	98±2
30	8±8	94±4

[a]The animals were re-infested after counting on day 10.
[b]Same group as treated dogs and cats as in **Table 1**.
Numbers are means ±SEM of the percent eradication (efficacy) of Solpreme at various intervals during the 30 days after infestation and treatment.
There were no statistical differences between groups (ANOVA).

All animals of all treatment groups showed normal health as monitored using the following criteria I. Body weight, II. Repeated veterinary examinations, III. Blood tests.

I. Body weight: One indication of wellbeing and health after treatment is body weight which was not reduced, and in fact there was a small increase in body weight from the beginning to the end of testing in almost all groups (**Table 3**.)

Table 3: The effect of a 30-day treatment with Solpreme anti-flea mixture on the body weight of dogs and cats: comparison between dogs and cats infested with fleas (infested) with their respective infested groups treated with Solpreme (Infested-Treated). Numbers are the mean present body weight change/100 (±SEM) between the first and the last day of the experiment.

Species	Treatment (n)	Body weight change
Dogs	Infested (4)	0.63±0.19
	Infested-Treated (44)	0.50±0.06
Cats	Infested (6)	0.00±0.06
	Infested-Treated (41)	0.11±0.03

II. Veterinary examinations: The results of the repeated veterinary examination were normal.

III. Blood tests: Standard battery of veterinary blood tests was conducted. All parameters urea, creatinine, bilirubin, *alkaline phosphatase (*ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were well within normal range.

Safety tests for the anti-fleas treatment mixture: The safety test of the anti-fleas mixture included: treating a group of dogs and cats with 5 times higher and lower than the recommended dose. The safety test of the non-active ingredients included: 5 times the recommended dose of control vehicle treatment that contains non-active, inert ingredients. Both did not show any adverse effect on the tested dogs and cats.

IV. DISCUSSION

The present study confirms the efficacy and safety of the mixture Dinotefuran, a new generation pesticides and the synergist piperonyl butoxoide, in the concentration developed in Solano laboratories (Solpreme Dog™ and Solpreme Cat™) in exterminating the fleas *Ctenocephalides canis* and *Ctenocephalides felis* in dogs and cats, using the Spot On application method.

While the combination of dinotefuran and piperonyl butoxide was tried for the control of other pests, for example as an enhancer of the efficacy of deltamethrin in mosquito control [14], the combination of the two pesticides in the concentration of dinotefuran 26% w/v and piperonyl butoxide 6% w/v as is shown here is novel and has never been attempted commercially.

Generally, toxicants were used in doses that appeared much less toxic to human and to the treated animals compared to the pests, without consideration of possible long-term effects. An archetypal example is the organophosphate family whose long-term effect on multiple systems was discovered only after many years of use. The central nervous system is a prime example where the Organophosphate Induced Delayed Neuropathy (OPIDN) was demonstrated [15]. It is significant therefore, that long-term toxicity of dinotefuran is long considered minor [16].

Piperonyl butoxide's long-term neurotoxicity, especially in the low doses applied here, is slight and was considered as posing no neurological risk by the EPA [17].

Further consideration in using toxicants is their possible teratological effects [18], and in the present case of a nervous system toxicant, the neurobehavioral teratological effect [19, 20]. The neurobehavioral teratogenicity of old generation pesticides such as the organophosphate, chlorpyrifos is well established [21, 22]. In addition to being less toxic to mammals than previous generation anti-fleas agents [23], both dinotefuran [24] and piperonyl butoxide [25, 26] appear to be non-teratogenic. However, there are indications that dinotefuran [24] and piperonyl butoxide [25] could be neurobehavioral teratogens and consequently we do not recommend their use during pregnancy. Still, further investigation is now required to confirm this notion.

Pertaining to the issue of developmental toxicity is the mechanism by which piperonyl butoxide enhances the efficacy of various toxicants including dinotefuran, acting as a synergist by inhibiting the P450-microsomal oxidizing system, diminishing the pest's ability to metabolize dinotefuran [5, 6]. The alterations in the oxidizing system are expected to be transient [27]. However, prenatal alteration of this system, for example induction of the microsomal oxidizing system by exposure to phenobarbital, uniquely lasts long into adulthood [28] adding to its potential developmental neurotoxicity and further supporting the recommendation for avoiding the use of Solpreme during pregnancy whenever possible.

The distribution in the skin of pesticide administered topically to dogs was monitored in previous studies with ^{14}C labelling. Radioactivity was detected inside the superficial epidermis, hair follicles and sebaceous glands which explains the water resistance of pesticide applied by the spot on procedure [29]. Indeed, in the present study the Solpreme efficacy was not diminished by repeated washing of the treated dogs.

Criteria for shelf life of pesticides are clearly defined (J. Capizzi, OPEW (Vol. XI, No. 3). Data from several laboratories is available on the shelf life of dinotefuran [17] and piperonyl butoxide[30] and its adequacy is now recognized by the EPA. The present study demonstrates that the mixture of the two substances in the concentration presented here, containing the non-active, inert ingredients possess shelf life of at least three years making it practical for veterinary use.

Solpreme was proven in the present study to be an effective treatment against *Ctenocephalides canis* and *felis felis* and still, safe for the dog and cat hosts. This was attested to by standard veterinary examinations, blood tests and monitoring body weight during the experimental period. Interestingly, body weight actually increased during the study, and this could be attributed to the continuous development of the young animals and to the weight increase with age in the adult animals.

In summary, we used a combination of dinotefuran, a relatively safe and effective pesticide with proven efficacy in the extermination of *Ctenocephalides canis* and *Ctenocephalides felis felis* and piperonyl butoxide, a known pesticide synergist in our established concentrations for the extermination of the fleas in dogs and cats. The treatment is shown to be effective, safe, water (wash) resistant and to have a long shelf life. Currently, it appears as an effective mean in the struggle with the continuous development of resistance to pesticides among these skin parasites.

Conflict of interest

J Yanai was employed by the Hebrew University-Hadassah Medical School, Israel. A Kafri was employed by Solano, Israel.

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REFERENCES

[1] T.B. Coles, M.W. Dryden, Insecticide/acaricide resistance in fleas and ticks infesting dogs and cats, Parasit Vectors, 7 (2014) 8.

[2] P.M. Linardi, G.L.C. Santos, Ctenocephalides felis felis vs. Ctenocephalides canis (Siphonaptera: Pulicidae): some issues in correctly

identify these species, Revista Brasileira de Parasitologia Veterinária, 21 (2012) 345-354.
[3] T. Wakita, K. Kinoshita, E. Yamada, N. Yasui, N. Kawahara, A. Naoi, M. Nakaya, K. Ebihara, H. Matsuno, K. Kodaka, The discovery of dinotefuran: a novel neonicotinoid, Pest Manag Sci, 59 (2003) 1016-1022.
[4] M. Tomizawa, J.E. Casida, Neonicotinoid insecticide toxicology: mechanisms of selective action, Annu Rev Pharmacol Toxicol, 45 (2005) 247-268.
[5] A. Tozzi, A Brief History of the Development of Piperonyl Butoxide as an Insecticide Synergist, in: D.G. Jones (Ed.) Piperonyl Butoxide: The Insecticide Synergist, Academic Press, San Diego, 1999, pp. 1-5.
[6] E. Hodgson, P.E. Levi, Interactions of piperonyl butoxide with cytochrome P450, in: D.G. Jones (Ed.) Piperonyl butoxide: The insecticide synergist, Academic Press, San Diego, 1998.
[7] A.W.A. Brown, R. Pal, Insecticide resistance in arthropods, Monograph Series, WHO, Geneva, 1971.
[8] W.H. Organization, W.E.C.o.V.B.a. Control, Vector Resistance to Pesticides : fifteenth report of the WHO Expert Committee on vector biology and control, World Health Organization, Geneva, 1992.
[9] R. Schenker, O. Tinembart, E. Humbert-Droz, T. Cavaliero, B. Yerly, Comparative speed of kill between nitenpyram, fipronil, imidacloprid, selamectin and cythioate against adult Ctenocephalides felis (Bouche) on cats and dogs, Vet Parasitol, 112 (2003) 249-254.
[10] M.K. Rust, Insecticide Resistance in Fleas, Insects, 7 (2016).
[11] M.J. Kotchen, Incorporating resistance in pesticide management: a dynamic regional approach, in: I. Ring, B. Klauer, F. Watzold, B. Mansson (Eds.) Regional Sustainability: Applied Ecological Economics Bridging the Gap Between Natural and Social Sciences, Springer Verlag, New York, 1999.
[12] M. Murphy, C.A. Ball, S. Gross, Comparative in vivo adulticidal activity of a topical dinotefuran versus an imidacloprid-based formulation against cat fleas (Ctenocephalides felis) on cats, Vet Ther, 10 (2009) 9-16.
[13] R.R. Sokal, F.J. Rohlf, Biometry, IV ed., Freeman, W. H., New York, 2011.
[14] F. Darriet, F. Chandre, Combining piperonyl butoxide and dinotefuran restores the efficacy of deltamethrin mosquito nets against resistant Anopheles gambiae (Diptera: Culicidae), J Med Entomol, 48 (2011) 952-955.
[15] M.B. Abou-Donia, Organophosphorus ester-induced delayed neurotoxicity, Annu Rev Pharmacol Toxicol, 21 (1981) 511-548.
[16] P.H. Rose, Nicotine and the Neonicotinoids, in: T.C. Marrs (Ed.) Mammalian toxicology of insecticides, Royal Society of Chemistry, Cambridge, UK, 2012, pp. 184-220.
[17] EPA, Pesticide Fact Sheet. 7501C DOI (2004) 1-63.
[18] E. Ujhazy, M. Mach, J. Navarova, M. Dubovicky, Teratology on the crossroads: historical aspects and modern approaches, Neuro Endocrinol Lett, 33 (2012) 304-313.
[19] J. Werboff, J.S. Gottlier, Drug in pregnancy: behavioral teratology, Obstetrical and Gynecological Survey, 18 (1963) 420-423.
[20] J. Yanai, Neurobehavioral teratology, Elsevier Science, Amsterdam, 1984.
[21] H. Billauer-Haimovitch, T.A. Slotkin, S. Dotan, R. Langford, A. Pinkas, J. Yanai, Reversal of chlorpyrifos neurobehavioral teratogenicity in mice by nicotine administration and neural stem cell transplantation, Behavioural Brain Research, 205 (2009) 499-504.
[22] G. Turgeman, A. Pinkas, T. Slotkin, M. Tfillin, R. Langford, J. Yanai, Reversal of chlorpyrifos neurobehavioral teratogenicity in mice by allographic transplantation of adult subventricular zone-derived neural stem cells, Journal of Neuroscience Research, 89 (2011) 1185-1193.
[23] M. Tomizawa, J.E. Casida, Selective toxicity of neonicotinoids attributable to specificity of insect and mammalian nicotinic receptors, Annu Rev Entomol, 48 (2003) 339-364.
[24] L.P. Sheets, A.A. Li, D.J. Minnema, R.H. Collier, M.R. Creek, R.C. Peffer, A critical review of neonicotinoid insecticides for developmental neurotoxicity, Critical Reviews in Toxicology, DOI (2016) 153-190.
[25] T. Tanaka, A. Inomata, Effects of Maternal Exposure to Piperonyl Butoxide (PBO) on Behavioral Development in F1-Generation Mice, Birth Defects Research (Part B), DOI (2015) 227-237.
[26] G. Kennedy, S.H. Smith, F.K. Kinoshita, M.L. Keplinger, J.C. Calandra, Teratogenic evaluation of piperonyl butoxide in the rat, Food and Cosmetics Toxicology, DOI (1977) 337-339.
[27] A.H. Conney, Conney, A. H. Pharmacological implications of microsomal enzyme induction, Pharmacological Reviews 19 (1967) 317-366.
[28] J. Yanai, Long-term induction of microsomal drug oxidizing system in mice following prenatal exposure to barbiturate, Biochem Pharmacol, 28 (1979) 1429-1430.
[29] H. Chopade, D. Eigenberg, E. Solon, P. Strzemienski, J. Hostetler, T. McNamara, Skin distribution of imidacloprid by microautoradiography after topical administration to beagle dogs, Vet Ther, 11 (2010) E1-10.
[30] W. Panel of Experts FAO, Toxicological evaluations, Pesticide Residues in Food, Geneva, Switzerland, 2011, pp. 607-705.

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